ONLINE METHODS

Strains. *C. elegans* strains were maintained under standard conditions[1], unless otherwise indicated. Wild-type worms were Bristol strain N2. Germline transformations were implemented as described[31]. Extrachromosomal array *sraEx47* was integrated into chromosomes using γ-ray irradiation. The resulting integrated strains were backcrossed to the N2 at least 8 times. Standard crosses were used to transfer the extrachromosomal arrays *sraEx80* and *sraEx83* into different genetic backgrounds. The success of genetic crosses was checked using the hydrophobic cyanine dye DiI staining of amphid neurons (*osm-3(p802)*), PCR (*tdc-1(n3419)*), blue-light avoidance assay (*lite-1(ce314)*), monitoring pharyngeal pumping rate (*eat-4(ky5)*) and osmotic drop assay (*eat-4(ky5), osm-3(p802)* and *osm-9(ky10)*). The genotypes used are listed as follows: N2 (wild type), *eat-4(ky5)*, *lite-1(ce314), osm-3(p802), osm-9(ky10)* and *tdc-1(n3419)*. Transgenic strains generated during this work are listed in **Supplementary Table 1**.

Molecular biology. *chop-2(H134R)::mCherry* was obtained by swapping mCherry with YFP in *chop-2(H134R)::YFP*[15]**.** We used a 5.1-kb *nmr-1* promoter that had been previously described[28]. We amplified 4-kb *sra-6*, 4.4-kb *tdc-1*, 0.8-kb *unc-122* and 2.9-kb *F55B11.3* promoters by PCR from *C. elegans* genomic DNA. All the promoters were cloned into the Fire lab vector kit plasmid pPD95.75 in which the sequence encoding GFP was replaced either by the sequence encoding G-CaMP or by *chop-2 (H134R)::mCherry*. The homozygous deletion of *tdc-1* was checked by PCR amplification. Primers used for PCR are listed in **Supplementary Table 2**.

Intensity measurement. A Newport 841-P USB power meter was used to measure the power of blue light reaching the Nematode Growth Medium (NGM) cultivation plate in each assay. The intensity of light reaching the culture plate for behavior assay was controlled using a combination of the click stop iris on the X-Cite Illuminator and the iris on the ZEISS Discovery dissecting microscope. The intensity used for behavioral assays (**Figs. 2**,**6** and **Supplementary Fig. 12**) was 10 mW mm^{-2}. To measure the intensity of the exciting blue light from lambda DG-4, a region was specified by DLP mirror array and the total power was used to calculate the intensity for stimulation. The intensity used for ChR2 stimulation was 8.0 mW mm^{-2}, which is comparable to the intensity used for behavioral assays (**Supplementary Fig. 17**). The intensity of the 488-nm laser used to monitor G-CaMP fluorescence was about 0.1 mW mm^{-2}, unless otherwise mentioned.

Behavioral assays. ChR2 requires the cofactor all-*trans*-retinal (ATR) for function. An overnight LB culture of *Escherichia coli* (strain OP50) was concentrated 20 times and mixed with ATR (Sigma) for a final concentration of 100 μM. We spread 300 μl of this bacteria-ATR suspension onto NGM plates. Mid-L4 larval worms expressing appropriate *mCherry* markers were selected the day before the experiment and cultivated on the newly seeded NGM plates overnight. A motorized ZEISS Discovery dissecting microscope equipped with a 120 W X-Cite lamp was used for all the avoidance behavior assays. An EGFP filter 480/40 nm was used to filter blue light for illumination. Young adult worms, slowly moving forward on the cultivation plate, were illuminated with blue light for about 1 s. Worms initiating backward movement during or within 1 s after illumination were scored as 1. Worms that accelerated forward movement, paused or initiated backward movement after 1 s of the end of illumination were scored as 0. The response of typically 30 worms was averaged to obtain the fraction of worms responding to blue light.

Statistical analyses. The standard chi-square test was used to determine the significance of the data for behavioral assays shown in **Figures 2**, **6** and **Supplementary Figure 12**. Welch's *t*-test was used to compare (i) the activation level in ASH and AVA in wild type, *lite-1, osm-3* and *eat-4* genetic background during ASH stimulation; (ii) the activation level in AVA during spontaneous reversals and during ASH or RIM stimulation; (iii) the activation delay in AVA in wild-type, *lite-1, osm-3* and *eat-4* genetic background (**Supplementary Note**). The amplitude of G-CaMP fluorescence fold change due to ASH or RIM neuron stimulation is defined as the maximum fold change after start of stimulation.

Optical stimulation and calcium imaging. Young adult worms were treated with 10-mM muscimol on a hydrated agar pad for imaging; muscimol, a GABA agonist, halts muscle contraction and worms developed a flaccid paralysis[32]. Immobilizing worms using muscimol was more convenient and typically gave more robust and reproducible optical signals compared to immobilizing worms using glue in our hands. For each stimulation experiment, a control experiment was performed with the activating light turned off, to show that there was no detectable G-CaMP fluorescence change during measurement. Then ChR2-expressing neurons were specifically stimulated while calcium activities in G-CaMP expressing neurons were monitored. Control measurements were also performed on worms grown without the ChR2 cofactor ATR.

Optical stimulations and recordings were performed on a Zeiss 200M inverted microscope. The microscope and all the other components were controlled by Metamorph software (version 7.5.3, Molecular Devices). Objective used was a ×63, NA = 1.4 Plan-Aprochromat oil objective. Neurons expressing *chop-2 (H134R)::mCherry* were identified using a Hamamatsu Orca-ER digital camera (C4742-80-12AG). Emission of mCherry was visualized at 650 nm (75-nm bandwidth) upon excitation at 565 nm (55-nm bandwidth). A high-speed Yokogawa CSU10 spinning disk confocal system equipped with DM488_BF488 filter set was used for imaging calcium activity and G-CaMP fluorescence was collected at 2 frames s^{-1} using a Hamamatsu electron-multiplying charge-coupled device (EM-CCD) digital camera (C9100-13). A digital-light-processing mirror array (Photonics Instrument) was used to generate a spatial mask that was subsequently used to deliver light for stimulation. A Lambda DG-4 optical switch equipped with a 300-W xenon lamp (Sutter Instrument) was used to deliver blue light, filtered with a HQ450/50X (Chroma) set, to activate ChR2. The power of the excitation light intensity from the DLP mirrors (8 mW mm^{-2}) was comparable to the excitation intensities where about 90% of the worms showed a behavioral response (**Supplementary Fig. 17**). A 475-nm long-pass filter was used to reflect blue light up to the objective for stimulation. For stimulating and imaging neurons on different focal planes, a NanoscanZ piezo stage system (Prior Scientific) was used to switch between different focal planes within 10 ms.

Calcium imaging data analysis. Images stacks (TIFF files) were analyzed by custom written scripts for Matlab (Mathworks). For each neuron, an approximate region of interest was defined by a rectangular region of 10 μm × 10 μm surrounding that neuron. The exact size of the region of interest was slightly adjusted to exclude the adjacent neurons. The fluorescence intensity within the region of interest F, as described[33], was calculated as the mean intensity of the brightest 128 pixels (~8 μm^2) minus the mean intensity of a background region that was far away from the body. The average fluorescence intensity within 2 s before stimulation was taken as the basal signal (F_0). The percentage change in fluorescence intensity for the region of interest relative to the initial intensity F_0, $(F - F_0)/F_0 \times 100\%$, was plotted as a function of time for all the activation experiments. Because the excitation spectra of ChR2 and G-CaMP overlap, blue light used for stimulating ChR2 neurons also excites G-CaMP, which produced a jump in G-CaMP fluorescence intensity (**Fig. 4b** and **Supplementary Fig. 18**). This immediate jump in fluorescence is directly proportional to the fold change in the illumination intensity of the neuron that is excited (since in the range of power intensities we operate, the signal from G-CaMP is proportional to the power of the excitation light). During the period of stimulation, the G-CaMP signal is amplified (compared to when there is no stimulation) by a constant factor that is equal to the fold change in the illumination intensity. To eliminate the effects of this jump we divide the G-CaMP fluorescent signal during the period of excitation by the magnitude of this initial jump. The photobleaching of G-CaMP over the duration of the light stimulation (10 s), due to the stimulating light, was 1.2 ± 0.8% ($n = 6$, tested on worms fed without retinal) and thus was not corrected. The photobleaching of G-CaMP during measurement was even smaller and within measurement noise. Different numbers of pixels, 256 and 64, were also chosen to calculate the percentage change of fluorescence intensity and the results were not different from using 128 pixels (**Supplementary Fig. 19**). Thus, 128 pixels were used unless otherwise specified. Schematic guides to **Supplementary Movies 3**, **4** and **6** are shown in **Supplementary Figure 20**.

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